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TITLE: Methods for cancer prognosis and diagnosis

Drawing Description Text (4):

FIG. 3 is a graph showing drug sensitivity in a HER2-sorted population of SKBR3 cells as determined by EDR assay and cytophobic culture plate assay.

Detailed Description Text (10):

The practice of one embodiment of the invention is shown in FIG. 1. A tumor sample or a tumor cell line is harvested and pure cancer cell population obtained by FACS sorting using fluorescently-labeled antibodies specific for neoplastic cell markers (such as HER2, EGFR or MDR1). The sorted pure cancer cell population is then expanded by growth in cell culture to provide sufficient cells for separation into drug-sensitive and drug-resistant populations. Drug resistant cells are separated from drug sensitive cells by culture in increasing concentrations of cytotoxic drugs, and the degree of drug resistance quantitated by growing the cells in a cell proliferation-specific detectable label (such as tritiated thymidine) for a terminal portion of each cell culture experiment. IC_{sub.50} values can be established by performing this assay in cytophobic plates that inhibit cell attachment (and therefore prevent proliferation of non-neoplastic cells). Finally, cell culture at the IC_{sub.50} concentration of the cytotoxic drug in cytophobic plates is used to prepare neoplastic cells for flow sorting. It will be recognized that a significant advantage of these methods is that a mixed population of drug-sensitive and drug-resistant cells are treated simultaneously under exactly identical conditions of cell culture and drug treatment and then analyzed after separation based on their differential drug resistance characteristics.

Detailed Description Text (23):

To approximate in vitro conditions for the growth neoplastic cells to the in vivo growth environment, Ultra Low Attachment 24-well plates (Costar, N.Y.) comprised of a covalently bound hydrogel layer that is hydrophobic and neutrally charged. This hydrogel surface inhibited non-specific immobilization of anchorage-dependent neoplastic cells via hydrophobic and ionic interactions and created an in vitro environment for culturing sorted and expanded neoplastic cells in organoid cultures. In titration experiments, the SKBR3 cell line (human breast cancer cell line, obtained from the American Type Culture Collection, Manassas, Va.) was plated in 24-well cytophobic plates at 500,000 cells per well, in duplicate, and treated with doxorubicin at concentrations of 0.02 .mu.M, 0.04 .mu.M, 0.08 .mu.M, 0.17 .mu.M, and 0.34 .mu.M in complete medium for 5 days. As with the EDR assay described above, cells were pulsed with tritiated thymidine at 5 .mu.Ci per well for the last 48 hours of the culture period and harvested with a micro-harvester. PCI was determined by comparing thymidine incorporation by the treatment group with incorporation by the negative control group (see section "EDR Assay"). Essentially the same experimental design was utilized to evaluate doxorubicin resistance of a fresh breast carcinoma sample # 80060899, using doxorubicin concentrations as follows: 0.001 .mu.M, 0.0025 .mu.M, 0.005 .mu.M, 0.01 .mu.M, 0.02 .mu.M, 0.04 .mu.M, 0.085 .mu.M, and 0.17 .mu.M.

Detailed Description Text (24):

To assess apoptotic potential of the SKBR3 cell line, SKBR3 cells at 500,000 per well were treated with doxorubicin at a pre-determined IC₅₀ (0.04 μ M) and IC₉₀ (0.34 μ M) concentrations for 24 and 48 hr, and analyzed by flow cytometry for Annexin V binding. In sorting experiments involving the SKBR3 cell line or sorted and expanded tumor populations, 3-5. \times 10⁷ cells were plated in cytotoxic 24-well plates at 500,000 cell per well, exposed to doxorubicin for 24 hours, collected by pipetting, washed with PBS with 1% FCS, and sorted on the basis of Annexin V binding.

Detailed Description Text (32):

FIG. 3 shows a comparison of doxorubicin sensitivity in a HER2-sorted SKBR3 cell population such as the one shown in FIG. 2C as determined by EDR assay and cytotoxic culture plate assay. Although the absolute IC₅₀ values differ, due to inherent differences in the techniques, the shape of the drug sensitivity curves are substantially parallel, indicating that cells exposed to cytotoxic drugs using the cytotoxic plate assay are equivalent to cells assayed using the EDR assay. This is important because cells assayed by the EDR assay are unsuitable for RNA isolation and gene expression analyses.

[Previous Doc](#)

[Next Doc](#)

[Go to Doc#](#)